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(21) International Application Number: PCT/US98/14000 (22) International Filing Date: 2 July 1998 (02.07.98) (30) Priority Data: 60/051,601 2 July 1997 (02.07.97) US (71) Applicant (for all designated States except US): EURO-CELTIQUE, S.A. [LU/LU]; 122, boulevard de la Petrusse, L-2330 Luxembourg (LU). (72) Inventors; and (75) Inventors/Applicants (for US only): GOLDENHEIM, Paul [US/US]; 4 Bald Hill Place, Wilton, CT 06897 (US). LACOUTURE, Peter [US/US]; 16 Ashford Lane, Newton, CT 06470 (US). DONIGI-GALE, Donna [US/US]; 22 Fieldcrest Drive, Richfield, CT 06877 (US). CHASIN, Mark [US/US]; 3 Wayne Court, Manalapan, NJ 07726 (US). SACKLER, Richard [US/US]; 25 Windrose Way, Greenwich, CT 06830 (US). (74) Agents: DAVIDSON, Clifford, M. et al.; Davidson, Davidson & Kappel, LLC, 15th floor, 1140 Avenue of the Americas, New York, NY 10036 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report:</i>
(54) Title: PROLONGED ANESTHESIA IN JOINTS AND BODY SPACES (57) Abstract <p>Sustained release local anesthetic formulations are administered intra articularly and/or into body spaces/cavities. The formulation is preferably a plurality of injectable microparticles including a local anesthetic and an effective amount of a biocompatible, biodegradable, sustained release material prolonging the release of the local anesthetic and optionally a pharmaceutically acceptable, i.e., non-toxic, augmenting agent effective to prolong the duration of the local anesthesia for a time period longer than that obtainable without the augmenting agent.</p>		

microspheres containing added ephedrine, in a percent loadings of 0.001 percent, 0.05 percent and 1 percent, without bupivacaine, are also prepared according to EXAMPLES 1-3 or EXAMPLES 4-9, above.

Following the protocol set forth in EXAMPLE 10, above, selected rats are injected adjacent to the sciatic nerve with a solution containing a suspension of bupivacaine-loaded microspheres on the right side, and on the left side with a solution containing a suspension of bupivacaine-loaded microspheres and also containing ephedrine containing microspheres in each dose level.

Sensory and motor testing is conducted according to sections A and B, respectively, of EXAMPLE 10, above. Using the experimental protocol of section C of EXAMPLE 10, 24 rats are tested for each of the three ephedrine dose levels by injecting ephedrine-containing microspheres (same number of microspheres per rat, adjusted for animal weight) at about the same time as the bupivacaine-containing microspheres are administered. On the sides receiving a combination of bupivacaine microspheres and ephedrine microspheres, a significantly longer duration of sensory block and significantly increased duration of motor block was obtained than with the sides receiving sustained-release bupivacaine-loaded microspheres without sustained release ephedrine for each dose level, with the effect showing a dose-response curve according to concentration.

EXAMPLE 16: IN-VIVO INJECTION INTO JOINTS

As can be appreciated, a substantial range of pharmaceutical agents is capable of augmenting the duration of local anesthetic activity. In addition, these compounds were tested as additives in the vehicle suspending the microspheres. Including an augmenting agent into the sustained release formulation itself is expected to substantially improve the prolongation of local anesthetic activity by prolonging the presence of augmenting agent at the anesthetized site.

EDLA (Extended Duration Local Anesthetic), as the term is used in this example, is a formulation of bupivacaine and dexamethasone in a matrix of poly(lactide:glycolide) 65:35 microspheres, which releases the bupivacaine and dexamethasone over a period of several days. The polymer is also biodegradable, and

Animals

The experiment was performed using three elderly male baboons that had previously been used in abdominal surgery studies, and which were scheduled to be sacrificed.

Protocol

The objective of the experiment was to inject EDLA microparticles into the knee joints of adult baboons and measure plasma concentrations of bupivacaine for several days, observe the movements of the animals for several days for induced lameness, physically examine the joints weekly for evidence of inflammation, and necropsy the animals and examine the joints grossly and histologically for lesions. Two animals received an injection of EDLA microspheres in one knee and vehicle in the other, while one animal received an injection of vehicle in one knee and no injection in the other knee (Table 4). The protocol was conducted in the following sequence.

Week 1, the animals were on a sham tether.

Week 2, the animals were on a tether.

Week 3, the knee joints were X-rayed and EDLA microspheres (bupivacaine and dexamethasone) were injected intra articularly.

During weeks 4, 5 and 6 serum drug levels, daily observations of walking were taken and physical examinations of the joints were conducted weekly. Final X-rays and necropsy was conducted at day 21.

TABLE 4
Administration of Test Substances

5	Animal	Joint space	Test substance
	A	Left knee	EDLA, 70 mg in 1 ml
		Right knee	Vehicle, 1 ml
	B	Left knee	Vehicle, 1 ml
		Right knee	EDLA, 70 mg in 1 ml of vehicle
10	C	Left knee	No injection
		Right knee	Vehicle, 1 ml

The animals were tethered so that frequent blood samples could be drawn during the first week after injection to measure plasma bupivacaine. Radiographs were taken prior to the injection of the test article and three weeks after injection (prior to necropsy) for evidence of lesions. The radiograph (X-rays) were confirmed by pathology studies, including gross and histologic evaluations.

B. RESULTS

1. In-life observations

No evidence of inflammation, tenderness or altered range of motion on weekly physical examination of knees, in any animal.

2. Plasma bupivacaine

Figure 2 is a graph which depicts the presence of plasma bupivacaine after the administration of the EDLA bupivacaine microspheres.

As shown in figure 2, Animal A, who was injected with 70mg of the EDLA in the left knee, had over 75 ng/ml plasma bupivacaine during day 1. Thereafter the level of bupivacaine decreased to approximately 60 ng/ml after day 2, and below 25 ng/ml after day 3, until the level reached 0 ng/ml after day 5.

Animal B, who was injected with 70 mg of the EDLA in the right knee had over 50 ng/ml plasma bupivacaine after day 1. Thereafter the level of bupivacaine decreased to 25 ng/ml after day 2 until the level reached 0 ng/ml after day 4.

Figure 2 confirms that Animal C, who was not injected with the EDLA, had no plasma bupivacaine during the days of the study.

3. Gross observations on necropsy

Joints were examined for swelling, warmth, and discoloration. All were negative. Range of motion and ease of motion were evaluated and all were judged normal. Joint capsule fluid was examined for transparency, color, and cells. All were negative. The joint capsule was examined for swelling and thickening, and for discoloration. All were negative. The cartilaginous surfaces of the medial femur, lateral femur, medial tibia, lateral tibia, medial meniscus, and lateral meniscus were examined for roughness. All were judged normal.

4. Histopathologic observations

The histopathological evaluations were conducted in a blinded manner.

Gross evaluation criteria were as follows.

Motion, mobility.

Inflammation was scored on a scale of 0-3 based on the observation of swelling, temperature and color.

Capsule fluid was scored on a scale of 0-3 based on transparency, bloody and purulent.

Joint capsule was scored on a scale of 0-3 based on swelling and color.

Cartilaginous surfaces were scored on a scale of 0-3, each, at the medial femur, lateral femur, medial tibia, lateral tibia, medial meniscus, lateral meniscus.

Results

All specimens were graded as "0".

Animal A: (EDLA, left knee): Giant cell formation around foreign material was evident in the synovial membrane of the left knee, with minimal lymphocyte infiltration around the giant cells. Cartilaginous surfaces were normal. Cartilage and synovial membrane was normal in the right knee. Diagnosis was listed:

5 Granulomatous arthritis, minimal, left knee.

Animal B: (EDLA, right knee): Giant cell formation around foreign material was evident in the synovial membrane of the right knee, with minimal lymphocyte infiltration around the giant cells. Cartilaginous surfaces were normal. Cartilage and synovial membrane was normal in the left knee. Diagnosis was listed:

10 Granulomatous arthritis, minimal, right knee.

Animal C (diluent, right knee): The cartilaginous surfaces and synovial membranes were normal in both knees.

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C. CONCLUSIONS

Injection of EDLA microspheres into the knee joints of normal baboons resulted in no damage to articulating surfaces when assessed after three weeks. EDLA particles were trapped in synovial membrane, with minimal foreign body reactions as a consequence. This type of reaction has been observed to EDLA and other microsphere formulations in most other studies. The incorporation of dexamethasone in EDLA microspheres results in an attenuated response; further increase in glucocorticosteroid concentration in EDLA microspheres may result in even less inflammation, and may itself provide a therapeutic benefit in osteoarthritic joints.

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Thus, the local tissue concentration of bupivacaine maintained by the EDLA microsphere formulation mirrors the observation that the local anesthesia produced by EDLA microspheres provides rapid onset and prolonged duration of action.

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EXAMPLE 17: IN-VIVO INTRAPERITONEAL ADMINISTRATION

In Example 17, a study was undertaken to examine intraperitoneal administration to rats. The goal of this exercise was to administer EDLA into another "cavity," the intraperitoneal cavity. The biological effect studied was inhibition of gastrointestinal motility as reflected by increased transit time through the small intestine. EDLA (low molecular weight polymer) was utilized in this study, and was prepared by forming an oil-in-water emulsion from an aqueous solution containing a surfactant (process water) and an organic solvent (oil) solution containing drug and polymer. Following emulsification, the solvent was removed in an aqueous quench allowing the microspheres to harden. Details are as follows:

Materials:

Process water (aqueous phase) was prepared as follows: A 1% stock solution of polyvinylalcohol (PVA) was prepared by the addition of 30 g PVA (Spectrum) to 3.0L of deionized water and heated while mixing to 65-70°C until dissolved. The PVA solution was cooled to ambient temperature and q.s. to 3.0L. Next, 375 ml of the stock PVA solution was diluted with 1125 ml of deionized water. Finally, 90 ml (80.1 g) of ethyl acetate NF (Fisher) was stirred into the process water prior to forming the emulsion.

The polymer/drug solution (organic phase) was prepared as follows: 5.6 g of Medisorb 65:35DL PLGA (inherent viscosity = 0.34 dl/g) was dissolved in 150 ml (133.5g) of ethyl acetate NF under ambient conditions. Next, 0.011 g dexamethasone (Upjohn) was added. Then, 14.4 g of bupivacaine base (Orgamol) was added to the polymer solution and sonicated until dissolved. Finally, the organic phase was filtered through a 0.22 μ m PTFE filter. The quench solution consisted of 8 L of deionized water at RT.

The organic phase and the aqueous phase were pumped simultaneously through a 1/2" diameter by 21 element static mixer (Cole Parmer) to form an emulsion. The organic phase was pumped at a rate of 500 ml/minute and the aqueous phase at 1000 ml/minute, into the quench solution, which was being stirred mechanically (500 rpm). The quench solution was then stirred for 1.5 hour, after

which the product was passed through 125 and 25 μm sieves. The 25-125 μm portion was collected on 10 μm filter paper and dried 4 hours under vacuum followed by air drying overnight. The process yield was 11.27 g of bupivacaine/dexamethasone-loaded microspheres (EDLA).

Method:

Charcoal, 10 % in gum acacia, 5 %, 0.25 ml, was administered to CD-1 mice by gavage. After 20 minutes, animals were euthanized using CO_2 . The gastrointestinal tract was removed, beginning with the stomach, carefully dissecting the mesentery to avoid stretching the small intestine, with transection at the ileocolic junction. The tract was measured from the jejunopyloric junction to the ileocolic junction. Finally, the distance traveled by the charcoal meal was measured. Gastrointestinal transit was quantitated as the percentage the meal traveled through the tract compared to the overall length of the tract.

In the trial experiment, a dose of EDLA (low molecular weight polymer), 50 mg in 0.3 ml, was administered 4 hours prior to the charcoal meal. In animals receiving vehicle, transit was $68 \pm 2 \%$, $n=10$, compared to $43 \pm 4 \%$, $n=10$ in the animals receiving EDLA, $t=5.66$, $df=18$, $p < 0.0001$.

EXAMPLE 18: IN-VIVO EPIDURAL INJECTION

In Example 18, the methods and procedures of Example 16 are repeated, except that the formulation is instead used for epidural administration. For epidural administration, a catheter is inserted, either for an acute administration or indwelling for chronic administration. The dose per administration should be 10-150 mg equivalent of bupivacaine, which is the maximum approved for the aqueous formulation. However, by virtue of the formulations used in the present disclosure, it has been demonstrated that such formulations are up to 40 times more safe than such approved formulations; therefore, it is possible that the dose of bupivacaine can be 40 times greater than the 10-150 mg equivalent cited above. The vehicle is the same used in other applications: sodium carboxymethylcellulose - 0.05%; polysorbate 80 - 0.1%; mannitol - 50 mM; pH 7.4. The EDLA microspheres are diluted so that

administration yields the desired bupivacaine dose in the desired volume (10 mg - greater than 150 mg) in 2 ml to 50 ml.

The examples provided above are not meant to be exclusive. Many other variations of the present invention would be obvious to those skilled in the art, and are contemplated to be within the scope of the appended claims. Numerous publications are cited herein, the disclosures of which are incorporated herein by reference in their entireties.